Luke Shively

Fish 460 Report

**Introduction**

Marine environments of today's Anthropocene are far from simple systems, with changes in climate, ecology, and economics driving the many ways we as humans are able to interact with them. Our interactions through the Alaskan snow crab fishery are an example of robust and rapid changes to marine environments completely shifting human markets, and driving further need for understanding more about the systems we extract so much from. The snow crab (*Chionoecetes opilio*) fishery in Alaska supported a near US$200 million industry with lucrative management processes and research conducted by the National Marine Fisheries Service. Despite these efforts, however, the Bering Sea snow crab population effectively disappeared from 2019-2021, and the reason why remains hidden beneath thousands of feet of water. What is known is that a marine heatwave swept through the North Pacific in the latter half of the 2010’s (Hu et al. 2024; any more?), which could have strained the snow crabs' thermal tolerance, causing either a mass migration to cooler northern or deeper waters, or metabolic hikes resulting in starvation (Szuwalski et al. 2023; ). Another factor speculated to have played a role in this event is tied to the phenomenon of bitter crab disease, a fatal dinoflagellate-borne illness with correlation to temperature increase and population density (Balstad et al. 2024; more). Ectothermic stress response can be significantly impacted by temperature increases (Shields 2019), and when critical temperatures are reached, the risk of infection and illness may pose a crucial threat to the immune response.

Understanding the immune response under increased thermal regimes can be done in the lab using a proxy for snow crabs. To do this, hairy shore crabs (*Hemigrapsus Oregonensis*) offer an opportunity to use an ecologically significant specimen that is malleable in a lab setting while still providing insight into the physiological responses of ectothermic decapods at local relevance. Hairy shore crab is a shore crab found along shorelines of the North American Pacific coast and is smaller than snow crabs, with roughly 30 mm wide carapaces and green-grey coloring (Dana, 18 CHECK IN). Hairy shore crabs have a larger thermal range, from 3°C-27°C, than snow crabs, with a thermal range of -1°C-5°C. The deep water environment in which snow crabs live makes for a less variable thermal regime, such that their tolerance has adapted to a narrow scope, whereas shore crabs and taxa that inhabit the intertidal are often at the hand of much more variable environments and thus have a much larger thermal scope. This more variable environment at the intertidal level also results in higher taxa richness of bacteria and parasites (Wang et al. 2012; MORE). Shields (2019) describes the lowered threshold to infection in crustaceans exposed to heightened temperatures, and Adamo (2012) outlines the lacking functions of the immune system compromised by infection as haemocyte count and

**Methods**

The experiment on hairy shore crab immune response as a response to heat stress was conducted over 3 weeks in the month of May, 2025, in the School of Aquatic and Fishery Sciences, Fisheries Teaching Building. H. oregonensis specimens were collected from mixed substrate (shell, sand, pebble) off of Lion’s Park Boat Launch, Bremerton, WA, between 11:30 and 13:34. Mud samples were collected from the streambed of a marginal freshwater outlet to Puget Sound near the shore within Golden Gardens Park, Seattle, Wa at 13:10.

4 groups were formed: a control group contained in cold water and no mud; one group contained in cold water with mud; one group contained in warm water and no mud; and one group contained in warm water with mud. With 6 crabs per treatment, excluding the control (18 total), 2 tanks (10 cm x 20 cm x 14.5 cm) containing mud were first hand-filled with 3.81 cm mud substrate and then filled with 1.5 L of water at an initial salinity of 33 ppt. 1 tank containing no mud was filled simply with 1.5 L of water at an initial salinity of 33 ppt. In all 3 tanks, 2 oyster shells serving as hides (15.96-33.6 cm^2) were placed in the tanks, one on either side of a mesh wall dividing the tank into two halves, with one side for unused specimens and one for used specimens. Non-manipulated control specimens were left in a large cool tank and not disturbed until measurements were made. Healthy, to-be-manipulated specimens, 6 per tank, were hand placed in each of the 3 tanks by hand. One crab in the cold water with no mud tank was missing a claw. Once tanks were set up, the two warm treatment tanks were set in a water table to be heated to 27°C, the one cold water treatment tank was set in another water table to be cooled to 13°C. Air stones supplied oxygen to each treatment tank for the duration of the study.

After one week of containment in respective treatments, measurements were collected for haemocyte count, respiration, glucose, and righting time. Haemocyte count was obtained by collecting haemolymph from crabs by way of syringical needle extraction at the arthrodial membrane in an optimally non-lethal procedure. Haemolymph was read using a haemocytomer to count the concentration of haemocytes. O2 respiration was obtained indirectly through metabolic rate by way of resazurin assays conducted following standardized protocol. As a proxy for respiration, fluorescence of stock solution in which the crab specimens are inoculated for 90 minutes is measured through the reduction of blue resazruin to red resarufin, read by a fluorescent spectrophotometer at 30 point intervals. Glucose was obtained through the haemolyph extractions and oxidized following Cayman Chemical protocol to produce hydrogen peroxide and gluconic acid. Hydrogen peroxide was then measured using peroxidase and a chromogen. Prior to each haemolymph extraction, crabs were submitted to a righting time test in which they were placed dorsal side down on a flat surface, and pressed down until release, at which point a stopwatch measured the amount of time it took for the crabs to reorient themselves. Haemocyte count, respiration, glucose, and righting time were collected two times per treatment one week apart. Crabs were to be used only once, and subsequently placed on the used side of their respective tanks for the remainder of the study. Once data was collected, simple statistical analysis was performed using Google Sheets to produce readings for the four metrics obtained.

**Results**

Haemocyte Count

5 specimens had haemolymph extracted as part of the first weeks measurement, while 5 speciments had haemolymph extracted as part of the second weeks measuremetns, including 2 from the control group. Haemocyte concentration was highest in the mud and heat treatment specimens with an average of 180 and 154 cells per microlitre in the first week, and a standard deviation of 90.24 and 43.13 respectively. In the second week, average cells per microlitre were 59.75 with a standard deviation of 6.56. In week 1, the mud and cold treatment had the second highest haemocyte concentration with an average of 42.50 and standard deviation of 15.93. The control treatment of no mud and cold had the third lowest haemocyte count with 18.75 and 5.5 cells per microlitre, and a standard deviation of 5.85 and 2.08 respectively. The no mud and heat treatment had the lowest haemocyte count in week 1 with an average of 13 and 5, with a standard deviation of 2.16 and 3.37 respectively. In week 2, the no mud and heat treatment had the second highest haemocyte count with cells per microlitre and standard deviation of 18.14. The mud and cold treatment had the third highest haemocyte count with an average of 29 cells per microlitre and a standard deviation of 12.25. The control group had the lowest average haemocyte count in the second week.

Respiration

The mud and cold treatment group had the highest average resazurin calculations (Figure 2.) Mud and cold individuals had an average RFU/g of 486.44 at 90 minutes with a standard deviation of 270.95 in week 1 and an average RFU/g of 495.81 in week 2 without a standard deviation due to there only being one measurement per treatment. No mud and heat individuals had the second highest average resazurin calculations with an average RFU/g in week 1 of 308.70 and standard deviation of 42.34. In week 2 the no mud and heat average RFU/g was 176.84 with no standard deviation again due to there only being one specimen per treatment. Third, the mud and heat treatment had an average RFU/g in week 1 of 243.66 with a standard deviation of 7.26 while week 2 had an average RFU/g of 157.39, once again, without standard deviation.

Righting Time

Righting time showed an overall increase from week 1 to week 2 (Figure 3.). Righting time for the control treatment was an average of 1.60 seconds. For the mud and cold treatment, righting time was an average of 0.89 seconds in week 1 and an average of 3.68 seconds in week 2. In the no mud and heat treatment, righting time was an average of 0.81 seconds in week 1 and an average of 1.28 seconds in week 2. Lastly, for the mud and heat treatment, righting time was an average of 2.78 seconds in week 1 and an average of 3.78 seconds in week 2.

Glucose

**Discussion**

A graph of a number of different sizes and colors

AI-generated content may be incorrect.A graph of a number of blue bars

AI-generated content may be incorrect.

B

A

Figure 1. (A) Average haemocyte count per treatment after one week (Control count collected with week 2 samples on 05/13/2025). (B) Average haemocyte count per treatment after two weeks (Control count collected with week 2 samples on 05/13/2025).

Figure 3. Righting time average across treatments for weeks 1 and 2 collected prior to haemolymph extraction/resazurin inoculation.

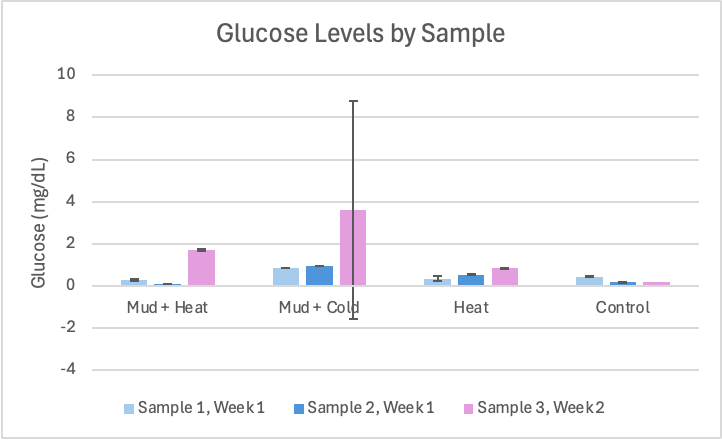


Figure 4. Glucose blah blah blah blah blah blah

Table 1. Average haemocyte counts for weeks 1 and 2 with standard deviation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Week 1 |  | Week 2 |  |
|  | Average Haemocyte Count | SD | Average Haemocyte Count | SD |
| Mud, 13 C | 42.5 | 15.92692 | 29 | 12.24745 |
| Mud, 13 C | - | - | - | - |
| No Mud, 13 C (Control) | - | - | 18.75 | 5.85235 |
| No Mud, 13 C (Control) | - | - | 5.5 | 2.081666 |
| Mud, 27 C | 180 | 90.24042 | 59.75 | 6.551081 |
| Mud, 27 C | 154 | 43.13545 | - | - |
| No Mud, 27 C | 13 | 2.160247 | 35.75 | 18.13606 |
| No Mud, 27 C | 5 | 3.366502 | - | - |

Hu, ZZ., McPhaden, M.J., Huang, B. *et al.* Accelerated warming in the North Pacific since 2013. *Nat. Clim. Chang.* 14, 929–931 (2024). <https://doi.org/10.1038/s41558-024-02088-x>

1.

WoRMS - World Register of Marine Species - Hemigrapsus oregonensis (Dana, 1851).<https://www.marinespecies.org/aphia.php?p=taxdetails&id=444778#attributes>.